Autoantibody in Mucous Membrane Pemphigoid Binds to an Intracellular Epitope on Human β4 Integrin and Causes Basement Membrane Zone Separation in Oral Mucosa in an Organ Culture Model

To the Editor:

Mucous membrane pemphigoid (MMP) is a rare vesiculobullous disease, which involves the oral, ocular, and other mucous membranes. Anti-basement membrane zone (anti-BMZ) autoantibodies with different specificities recognize different target molecules present in the BMZ, many of which have been recently described (Scully *et al*, 1999). A subset of MMP patients whose disease is limited to, or principally involves, the conjunctiva are known to have ocular cicatricial pemphigoid (OCP) (Foster, 1986).

In our earlier studies, we demonstrated that sera of patients with OCP bind to the human B4 molecule in human skin, conjunctiva, and tumor cell lysates (Tyagi *et al*, 1996; Chan *et al*, 1999). Our recent observations indicate that the OCP autoantibody recognizes only the intracellular domain of human β4 integrin and not the extracellular domain (Bhol *et al*, 2000). In subsequent studies we have identified that the antibody binds to a peptide (IC 3.4), within the intracellular portion of human β4 integrin (Kumari *et al*, 2001). The presence of intracellular antigen in human autoimmune disease has been described by several investigators (Alarcon-Segovia *et al*, 1978; Golan *et al*, 1992; Reichlin, 1995). In this study, we used several clones representing fragments of the intracellular portions of B4 (IC 1.0, IC 2.0, IC 3.0, IC 3.4) and tested their binding to sera from 10 MMP patients with multiple mucosal involvements, with active disease. All the MMP sera demonstrated binding to the BMZ of normal skin, conjunctiva, and buccal mucosa, and to the epidermal side of the salt split skin, by indirect immunofluorescence (indirect immunofluorescence titer ranged from 1:80 to 1:640), and bound to the recombinant fusion protein representing IC 3.0 and IC 3.4 fragments only, produced in *Escherichia coli*, in an immunoblot assay (**Fig 1*a***). These results indicated that, for MMP sera, the dominant antibody binding epitope is IC 3.4 (83 amino acids). Sera from rabbit immunized with IC 3.4 protein demonstrated binding to IC 3.4 fusion protein (**Fig 1**). Normal human sera, preimmune rabbit sera, and sera from patients with pemphigus vulgaris did not bind to this fragment of β4 integrin.

In this, our purpose was to determine the potential role of IC 3.4 in the pathogenesis of MMP and the ability of antibodies to IC 3.4 to produce separation of epithelial cells from the BMZ, in an organ culture model, using normal human buccal mucosa. Such studies have been done using normal human conjunctiva (Chan *et al*, 1999) but not with other mucosa. As oral mucosa is frequently involved in MMP, it was important to determine if the possible role for antibody to IC 3.4 was identical. Sera from 10 MMP patients produced BMZ separation, identical to that observed *in vivo*, as did rabbit antibodies to human IC 3.0 or IC 3.4 (**Fig 2**).

These observations strongly suggest that anti-BMZ antibodies in the sera of MMP patients target the same peptide in the intracellular portions of B4 (IC 3.4) as do autoantibodies in OCP. Hence these observations have wider implications. It is possible that a similar mechanism may involve other mucosae involved in MMP.

It has been reported that a different subset of MMP, involving multiple mucous membranes, produce antibodies to laminin 5 (Kirtschig *et al*, 1995). The sera of patients with antibodies to laminin 5 bind to the dermal side of the salt split skin. The sera of patients tested in our study do not contain autoantibodies to laminin 5 (Kumari *et al*, 2001) and bind to the epidermal side of the salt split skin.

As MMP has a heterogeneous clinical profile and clinical course, it would appear that there could be several antigens involved alone or in combination in generating or maintaining the autoimmune response. Some investigators have demonstrated binding of MMP sera to bullous pemphigoid (BP 180, BP Ag2). It yet remains to be proven, however, whether the antibody to BP Ag2 is the primary disease producing autoantibody or is produced as a secondary antibody. Neonatal BALB/c mice injected with antibodies to BP Ag2 do not develop mucosal disease.

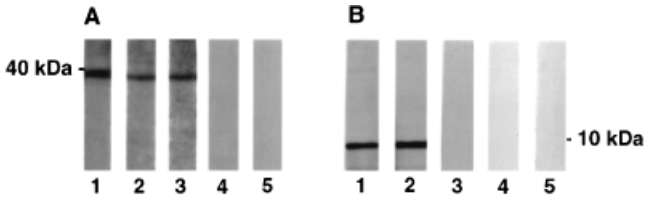

Figure 1. Binding specificity of sera to IC 3.0 and IC 3.4. Immunoblot analysis of fusion proteins produced by clones representing IC 3.0 and IC 3.4 fragments, presented in *panel A* and *panel B*, respectively. The fusion proteins in *lane 1* in both the panels are immunoblotted with MMP sera. Note an ≈37 kDa band indicating binding to IC 3.0, and an ≈10 kDa band indicating binding to IC 3.4 by MMP sera. The fusion proteins in *lane 2* in *panels A* and *B* are immunoblotted with rabbit antibody to IC 3.0 and IC 3.4 fragments, respectively. The presence of an ≈37 kDa band indicates binding to IC 3.0 and an ≈10 kDa band indicates binding to IC 3.4. The fusion proteins in *lane 3* in both the panels were immunoblotted with antibody to the cytoplasmic domain of β4 integrin. Note binding to IC 3.0 as demonstrated by the presence of an ≈37 kDa band, and absence of binding to IC 3.4. The fusion proteins in *lanes 4* and *5* in both the panels were immunoblotted with normal human serum and preimmune rabbit serum, respectively. Note absence of binding either to IC 3.0 (≈37 kDa) or IC 3.4 (≈10 kDa).

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Reprint requests to: A Razzaque Ahmed, M.D., Department of Oral Medicine and Diagnostic Sciences, Harvard School of Dental Medicine, 188 Longwood Avenue, Rm. I-217, Boston, MA 02115; Email: razzaque.ahmed@hms.harvard.edu

Abbreviations: MMP, mucous membrane pemphigoid; BMZ, basement membrane zone.

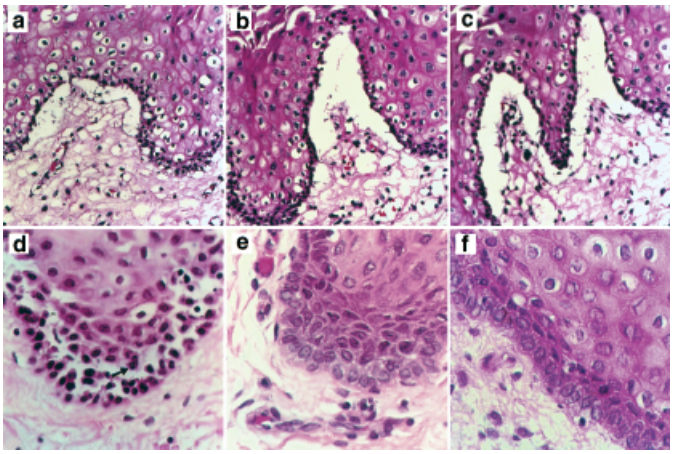

Figure 2. Organ culture model to demonstrate BMZ separation of buccal mucosa. *In vitro* organ culture model of normal human buccal mucosa incubated with (*a*) MMP serum, (*b*), (*c*) rabbit antibody to IC 3.0 and IC 3.4 fragments, respectively, (*d*) PV serum, (*e*), (*f*) normal human serum and preimmune rabbit serum, respectively. In the normal human buccal mucosa section cultured with MMP serum and rabbit antibodies to IC 3.0 and IC 3.4 histologic identical BMZ separation was observed. Pemphigus vulgaris serum produced acantholysis of epithelial cells in the buccal mucosa, serving as a positive control for the assay. Normal human serum and preimmune rabbit serum did not produce BMZ separation.

Hence the specific role for antibodies to BP Ag2 in the pathogenesis of MMP is not yet clear.

Investigators studying MMP may be able to obtain oral mucosa more easily than human conjunctiva. The availability of an organ culture model will permit rapid advances in understanding the multiple steps involved in the pathogenesis. It will also help us understand why certain mucosae are more frequently involved than others. If the multistep pathogenesis is delineated, attempts to arrest progression may be possible.

This model also provides an opportunity to study the mechanism by which autoantibodies that target intracellular antigens gain access to them. If this process is better understood, the model has the capacity for testing new biologic agents to arrest or prevent the progression of the disease process, if delivered locally or specifically targeted when administered systemically.

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K. C. Bhol, J. E. Colon, and A. R. Ahmed
Departments of Dermatology, Oral Medicine, Infection and Immunity, Harvard Medical School, and Harvard School of Dental Medicine, Boston, Massachusetts, U.S.A.

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